

SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: Dr. M. Michael Sigel

Date: July 27, 1953

NR: 130-706

For Period: 1/1/53 to
6/30/53

CONTRACT: N8onr-72601

PRESENT ANNUAL RATE:

CONTRACTOR: The Children's Hospital of Philadelphia

INVESTIGATORS: Dr. M. Michael Sigel

Dr. Louis Grant, University College of West Indies
Mona, Jamaica

Dr. Carroll F. Burgoon, Children's Hospital of Philadelphia
Philadelphia, Pennsylvania

Alan Bernstein

Miss Betty Bratton

TITLE OF PROJECT: "Studies on the Psittacosis-Lymphogranuloma
Venereum Group"

- Objectives:
1. To search for a specific complement fixing antigen. At present, there is no satisfactory serologic procedure available for differentiation between infections caused by members of this group.
 2. To study mode of growth and multiplication and other biologic characteristics of this group of agents. These studies may have a bearing on the problem listed under (1).

Furthermore, knowledge obtained from these studies may help define the position of these agents in the microbial world.

3. To study the effect of therapeutic agents (antibiotics) on these viruses and on the diseases caused by them.

Abstract (or Summary) of Results:

A. Since Start of Project:

The following phases of the work have been completed:

1. Studies on mode of multiplication of the psittacosis-lymphogranuloma venereum group of viruses in the chick embryo and in tissue cultures.
2. Studies on the effect of aureomycin on meningopneumonitis virus.
3. Studies on the preservation of viruses under various conditions of storage.
4. Preliminary investigations on the demonstration of the specific complement fixing component.

These results have already been presented in previous semi-annual reports, ONR Progress Report Abstract, and in the following papers already published or about to be published:

1. Studies on the psittacosis-lymphogranuloma group. I. The pattern of multiplication of meningopneumonitis virus in the allantois of the chick embryo. M. M. Sigel, A. J. Girardi and E. G. Allen. J. Exp. Med., 94, 401, 1951.

LEGIBILITY POOR

- 3 -

2. Studies on the psittacosis-lymphogranuloma group. II. A non-infectious phase in virus development following adsorption to host tissue. A. J. Girardi, E. G. Allen and M. M. Sigel. J. Exp. Med., 96, 233, 1952.
3. Studies on the psittacosis-lymphogranuloma group. III. The effect of aureomycin on the propagation of virus in the chick embryo. E. G. Allen, A. J. Girardi, M. M. Sigel and M. Klein. J. Exp. Med., 97, 783, 1953.
4. Distribution and persistence of aureomycin in the chick embryo. E. G. Allen, A. J. Girardi, M. M. Sigel and M. Klein. Proc. Soc. Exp. Biol. & Med., 82, 542, 1953.
5. Preservation of viruses of the psittacosis-lymphogranuloma venereum group and herpes simplex under various conditions of storage. E. G. Allen, B. Kaneda, A. J. Girardi, T. F. M. Scott and M. M. Sigel. J. of Bact., 63, 369, 1952.
6. Studies on the psittacosis-lymphogranuloma group. IV. Demonstration of specific CF antibodies following absorption of serum. R. Pollikoff and M. M. Sigel. (To be published).
7. The reduction of group reactivity of the complement fixing antigen of meningopneumonitis virus by potassium periodate. M. M. Sigel and R. Pollikoff. (To be published).

B. During Current Report Period:

Work on the Propagation of MT Virus in Tumor Cells

Further attempts were made to quantitate the interaction between MP virus and Krebs-2 ascites tumor cells. The results obtained differed in some respects from previous findings. The two sets of experiments were conducted in different strains of mice. It now appears that mice may differ with respect to their susceptibility to virus as well as to tumor cells. We are therefore contemplating parallel determinations in the two strains of mice as soon as the previously used strain becomes available again. Work was also continued on adaptation of tumor cells to growth in tissue culture. Thus far we have failed to grow the Krebs-2 and the Ehrlich tumor cells in vitro. Successful growth was obtained with the following types of cells: The Earl L cells, sarcoma 180 and HeLa cells.

Epidemiological Studies on Lymphogranuloma Venereum

In Philadelphia

In order to determine the importance of lymphogranuloma venereum as a public health problem a survey was carried out in two VD clinics in Philadelphia. Random samplings were carried out during several days. In all, 270 patients were tested. The results obtained are most revealing:

140 were negative for both LGV and syphilis

50 were positive for LGV and negative for syphilis

Of these, 23 were males, 26 were females and 1 on whom the sex was not

specified. This is interesting in view of the fact that the overt form of the disease is seen more frequently in the male than in the female.

7 patients had serologic reactions for both LGV and syphilis

10 had a positive reaction for LGV and doubtful for syphilis

3 had doubtful reaction for LGV and positive for syphilis

2 were doubtful by both tests

6 were doubtful for LGV and negative for syphilis

There were 30 individuals with positive serology for syphilis and 18 with doubtful serology for syphilis who had negative test for LGV. This attests to the relative specificity of the LGV test in syphilitic sera. It should also be mentioned that many of the sera were absorbed with sheep cells in order to remove Forssman type of antibody. There were 4 nonspecific reactions, 3 in people with syphilitic reactions. The positive results for LGV were based on a titer of 10 or higher and in order for a serum to be considered positive it had to give such titers on two occasions. The ages of the individuals ranged from 10 to 50 years with the majority being in the 20 to 40 age group. Among the 270 patients there were 34 of the Caucasian race. Among them, 29 were negative for both syphilis and LGV, 1 was doubtful for LGV, 2 were positive for LGV, and 2 were positive for syphilis and nonspecific for LGV.

The important finding is that even if one disregards the positive LGV reactions in those individuals with positive or doubtful syphilis reaction, there were 50 out of 270 individuals, or 18%, who showed evidence of previous infection with a member of the psittacosis-lymphogranuloma

venereum group of viruses - based on a titer of at least 1:10.

Joint Studies Carried Out in Jamaica and at Children's Hospital
of Philadelphia

Sera from 75 prostitutes in Jamaica were tested for antibodies to LGV, gonorrhea, and syphilis. The results with the GC antigen are difficult to interpret because specificity of the test was not ascertained. The test for syphilis in Jamaica was the VDRL test.

19 had a positive test for syphilis

27 had a positive test for LGV (Of these 16 had a negative test for syphilis, 5 had a doubtful test for syphilis and 6 were positive for syphilis.)

It may be important to stress that of the 27 reactors, 14 had a titer of less than 1:40.

9 had a titer of 1:40 or 1:80

3 had a titer of 1:160 - 1:320

1 had a titer of 1:640.

Complement fixation tests have been completed on the first year's blood specimens of the school children in several parts of Jamaica. As was stated in the summary of the initial phase of study in Jamaica: "Very little is known about the existence of psittacosis or trachoma in Jamaica. It was felt that continued examination of sera from children may throw some light on the question as to what proportion of LGV antibodies represents venereally acquired infections." These investigations cover children

starting with ages under puberty and continuing on for several years into the ages where sexual activity begins to develop. The sera were tested chiefly for antibodies to the LGV group of viruses. In addition, a number were examined for serologic evidence of infection with other viral and rickettsial agents. It may be said in passing that many reacted with influenza antigens and several reacted with neurotropic viruses and rickettsiae. We wish, however, to postpone final conclusions about the significance of the neurotropic virus and rickettsial findings until certain additional studies are carried out in order to assess the specificity of the results. As regards results obtained with the LGV antigen, they may be summarized in the following fashion:

- a. Calabar School, Kingston, Jamaica
102 sera tested - 1 positive
- b. St. Aloysius School, Kingston, Jamaica
18 sera tested - 0 positive
- c. St. Joseph School, Kingston, Jamaica
49 sera tested - 0 positive
- d. School in Vere
80 sera tested - 0 positive
- e. School in May Pen
71 sera tested - 0 positive
- f. School in Watermount
62 sera tested - 1 suggestive, 0 positive

It should be pointed out that Watermount is located in a yaws endemic area.

It is planned to obtain the second bleeding from all the children under study during the fall of 1953.

In order to further assess the specificity of the LGV tests, a number of sera from patients with yaws were tested in the complement fixation test.

70 sera tested - 8 positive

3 doubtful

Thus it appears that yaws does not cause a significant number of false positive reactions. It is not known at the present time what age groups these sera represented and it is thus impossible to state whether the few positive reactions reflect true infection with members of the psittacosis-lymphogranuloma venereum group of viruses or whether they are nonspecific reactions.

Similar studies were carried out on 14 sera from patients with leprosy. The following results were obtained:

2 gave nonspecific reactions

2 were anticomplementary

1 was positive

1 was doubtful

8 were negative.

Thus it appears that leprosy per se does not cause nonspecific reactions as determined with the Lygranum-Squibb antigen. However, the number is too small for definite conclusions.

Effect of Therapy on Lymphogranuloma Venereum

Thus far, 96 patients in Jamaica were treated with various drugs.

31 with Aureomycin

13 with Ilotycin

14 with Chloromycetin

5 with Magnamycin

8 with Terramycin

13 with Triple Sulfonamide

12 with Sulfathiazole

The initial response has been good except with Magnamycin. Patients receiving this drug had to be retreated with a different antibiotic. It is planned to increase all treatment groups.

Dr. Burgoon visited in Jamaica and checked on the progress of the work there in the month of May. He found the study progressing quite satisfactorily. We are extremely pleased with the work and cooperation on the part of the investigators in Jamaica. Serum specimens were collected from all the patients under treatment. The number of specimens per patient ranged from 1 to 6. It is hoped that the serologic pattern revealed by these tests will impart information as to whether or not intensive antibiotic therapy can influence antibody formation and as to whether or not clinical improvement (or cure) in LGV is associated with a decrease in antibody level. Thus far, it was possible to observe a decrease in antibodies in 10 individuals. This work is being continued and more illuminating information is anticipated when more patients and

especially when additional sera on the same patients are examined.

PUBLICATIONS DURING PAST 6 MONTHS:

1. Studies on the psittacosis-lymphogranuloma group. III
The effect of aureomycin on the propagation of virus
in the chick embryo. E. G. Allen, A. J. Girardi,
M. M. Sigel and M. Klein. J. Exp. Med., 97, 783, 1953.
2. Distribution and persistence of aureomycin in the chick
embryo. E. G. Allen, A. J. Girardi, M. M. Sigel and
M. Klein. Proc. Soc. Exp. Biol. & Med., 82, 542, 1953.
3. The mounting incidence of psittacosis. M. M. Sigel,
L. S. Cole and O. Hunter (Accepted for publication in
the Am. J. Pub. Health).
4. The reduction of group reactivity of the complement
fixing antigen of meningopneumonic virus by potassium
periodate. M. M. Sigel and R. Pollikoff. (To be
published)
5. Studies on the psittacosis-lymphogranuloma group. IV.
Demonstration of specific CF antibodies following
absorption of serum. R. Pollikoff and M. M. Sigel.
(To be published).

PLANS FOR THE FUTURE:

Investigations will be continued on a limited scale on the problem
of interaction between tumor cells and MP virus. The principal part of

the research will consist of continued epidemiologic, serologic and therapeutic investigations in collaboration with the University College of West Indies and the Medical Service of the Government of Jamaica.